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In vivo Metabolism, Disposition and Macromolecular Binding of 1-Nitro (14C) Pyrene Vapor-Coated onto Diesel Particles

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IN VIVO METABOLISM, DISPOSITION AND MACROMOLECULAR BINDING OF 1-NITRO [^{14}C] PYRENE VAPOR-COATED ONTO DIESEL PARTICLES

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16. ABSTRACT The potent mutagen and environmental pollutant 1-nitropyrene (NP) labeled with ^{14}C and vapour-phase-coated onto diesel particles (^{14}C-NP-DP; 380 ppm NP; 0.85 µCi/mg particles) was administered to rats (5 mg each) by oral or by intratracheal instillation. In both cases 20-30% of the ^{14}C was recovered in the urine and 40-60% in the faeces within 24 h. After intratracheal instillation the majority of the particles were recovered in the lung, trachea and oesophagus, while after oral dosing the particles were found mainly in the faeces. Free 6-and 8-hydroxy-N-acetyl-1-aminopyrene were found in the urines by HPLC analysis, ≈7 and ≈4% of the urinary ^{14}C respectively. This was somewhat increased after incubation with sulphatase, and reached ≈11 and ≈14% after incubation with β-glucuronidase. 6-Hydroxy-N-acetyl-1-aminopyrene was previously found to be the metabolite responsible for the majority of the mutagenic activity in the urines of rats injected with pure NP. Phenols of NP itself were also identified, both free and conjugated, representing 1-5% of the urinary ^{14}C. The extent of urinary excretion indicated that NP was readily bioavailable from the dose remained in the lung 24 h after intratracheal instillation, and 5 to 15% of that ^{14}C appeared to be bound to protein. Since NP has previously been shown to bind to lung DNA and protein in vitro, these results indicate that this organ might be particularly exposed in vivo and therefore vulnerable to possible genotoxic damage by NP. This is an abstract of a proposed presentation and does not necessarily reflect U.S. EPA policy.

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IN VIVO METABOLISM, DISPOSITION AND MACROMOLECULAR BINDING OF 1-NITRO[^{14}C]PYRENE VAPOR-COATED ONTO DIESEL PARTICLES

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INTRODUCTION

1-Nitropyrene (NP) has been detected in photocopier toners (1,2) and diesel emissions (3). Nitropyrenes in general are highly mutagenic (4); NP itself was moreover carcinogenic to rats (5,6). 1-Nitropyrene undergoes reductive and oxidative metabolism (Figure 1), and both routes have genotoxic implications. Mutagenicity and binding to DNA in Salmonella appear to be linked to production of an active electrophilic intermediate (arylnitrenium ion or hydroxylamine) during the process of nitroreduction (7,8). In vitro, mammalian metabolism produces phenols of NP which are themselves mutagenic to Salmonella (9,10). Studies of the metabolic fate of NP in vivo indicate that in the rat nitroreduction (carried out predominantly by the gut flora), ring oxidation (in the liver) and N-acetylation (possibly in the liver or the kidneys) and conjugation (in the liver) can all occur sequentially, leading to production of acetamidopyrenols which are potent S9-dependent mutagens and are excreted in the urine (11,12). 1-Nitro[^{14}C]pyrene was also found to bind to endogenous macromolecules (DNA and protein) of rabbit pulmonary tissues in vitro (13). From 2 to 5% of the DNA adducts formed by rabbit lung tissue appeared to be identical (14) to the C8-deoxyguanosine-aminopyrene adduct previously identified as the major adduct formed from NP by Salmonella typhimurium TA1535 and by xanthine oxidase (8).

Since NP occurs in the environment largely associated with combustion particulate, we needed to ascertain whether our previous findings [carried out with pure NP administered by intra-peritoneal injection(11,12)] were applicable when NP was present in the matrix of organics coated onto diesel particles, and administered in vivo by intratracheal instillation, which corresponds more closely to respiratory exposures. For this purpose we obtained some previously-characterised diesel particles and vapor-coated 1-nitro[^{14}C]pyrene onto them. This enabled us to compare the distribution, metabolic fate and

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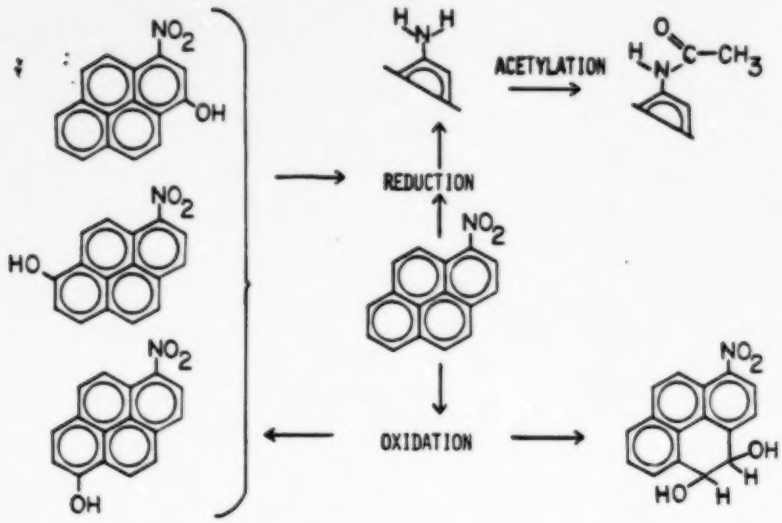

FIGURE 1. Metabolism of 1-nitropyrene: Oxidation produces 1-nitropyrene-4,5-dihydrodiol (Dihydrodiol, lower right) and 3-hydroxy-1-nitropyrene (3-OH-NP), 6-hydroxy-1-nitropyrene (6-OH-NP) and 8-hydroxy-1-nitropyrene (8-OH-NP) (left) which in vivo are reduced then acetylated (upper right) to 3-hydroxy-N-acetyl-1-aminopyrene (3-OH-NAAP), 6-hydroxy-N-acetyl-1-aminopyrene (6-OH-NAAP) and 8-hydroxy-N-acetyl-1-aminopyrene (8-OH-NAAP) respectively. Direct reduction of 1-nitropyrene (1-NP; center) forms 1-aminopyrene (1-AP) which can be acetylated (upper right) to N-acetyl-1-aminopyrene.

macromolecular binding of NP administered alone or adsorbed onto particles. We also instilled 1-nitro[^{14}C]pyrene simultaneously with unlabelled particles, to see how the presence of the particles in itself affects the disposition of NP.

MATERIALS AND METHODS

Chemicals

1-Nitro[4,5,9,10-^{14}C]pyrene (^{14}C-NP;60 mCi/mmole) and the NP phenol standards were prepared by the Midwest Research Institute, Kansas City, MO. These and other reference compounds are described in 10,12. Solvents were purchased from Burdick and Jackson, Muskegon, MI; enzymes for conjugate and DNA analysis, and other biochemicals, were from Sigma Co, St Louis,MO.

Vapor-Coating of Diesel Particles

The collection and characterization of the diesel particles have been described elsewhere (15). 1-Nitro[^{14}C]pyrene was vaporized at 275°C into a doubly-silated glass vessel into which the aerosolized diesel particles were delivered on a stream of N_2 gas. The particles were collected on a Pallflex filter then recovered by brushing off. This procedure is described more completely elsewhere (S. Lucas, Battelle Columbus Laboratories, Columbus, OH, Internal Report, EPA Contract No. 68-02-3310C9, and MS in preparation). The recovered particles were extracted with CH_2Cl_2 and the extracts analysed by HPLC and liquid scintillation counting (LSC) for total NP content (determined by fluorescence as 1-aminopyrene, [16]), and radiochemical content and purity, to verify that ^{14}C-NP had not undergone significant decomposition during the vapor-coating process. The particles were shown to contain 380 ppm of NP, and 195 000 dpm of ^{14}C per mg of particles, which consisted of > 99.5% ^{14}C-NP.

Animals

The ^{14}C-NP-fortified diesel particles and the unlabelled particles were suspended, and the ^{14}C-NP was dissolved, in phosphate-buffered saline containing 1% Tween 80. The doses were adjusted to yield 5 mg of particles and 8 nmol ^{14}C-NP in 0.2 ml, and administered to halothane-anaesthetised male rats (Sprague-Dawley, Charles River CD-1, Wilmington, MO; 5 mg each) by intratracheal instillation *via* a #18 curved needle. The rats were maintained in metabolism cages (Nalge Co., Rochester, NY), for separate collection of urine and feces, and allowed powdered food (Ralston Purina Co., St. Louis, MO) and drinking water *ad lib*. Twenty-four hours after dosing, the rats were anaesthetized with ether, exsanguinated and sacrificed. Lungs and other specific organs were dissected out separately.

Analytical techniques

The ^{14}C in urine and other fluid samples was determined by LSC. Small tissues were solubilized with Protosol (New England Nuclear, Boston, MA), and larger organs homogenized in lysing buffer (50 mM Tris-HCl, 10 mM EDTA, 1% SDS, pH 7.9) before LSC.

For HPLC analysis urine samples were concentrated on Sep-Pak® cartridges (Waters Associates, Milford, MA) then separated on a Varian Model 5060 HPLC, with a Zorbax ODS column, eluted with a gradient of MeOH in H_2O (see 12). Urine samples were analysed both before and after incubation with β-glucuronidase or sulphatase. Metabolites were identified by comparison with

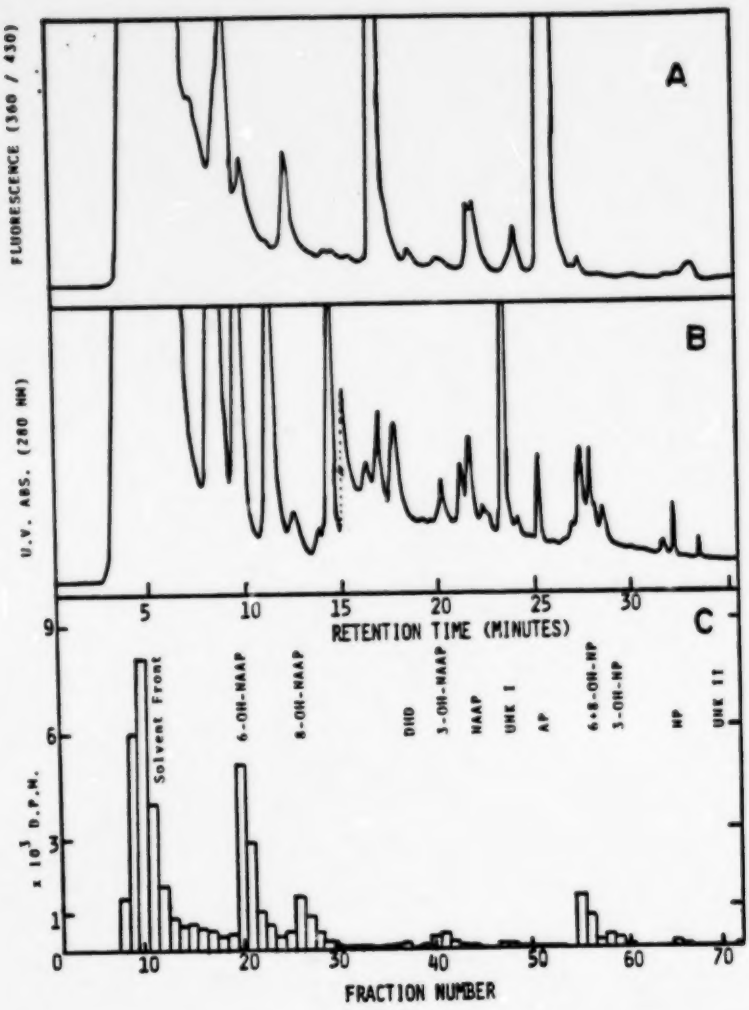

FIGURE 2. HPLC profile of metabolites and standards. Urine was collected over 24 h from a rat dosed intra-tracheally with 1-nitro[^{14}C]pyrene (8 nmoles, 0.5 µCi) vapor-coated onto diesel particles (5 mg), incubated with β-glucuronidase, concentrated, spiked with synthetic standards then analysed by reverse-phase HPLC. (A) Fluorescence, Excitation: 360 nm, Emission: 430 nm; (B) Absorbance at 280 nm; (C) ^{14}C, determined by LSC of fractions of the eluate collected every 30 sec. Metabolite structures and abbreviations of metabolite names are shown in Figure 1.

synthetic standards and with previously-identified metabolites (10,12). Figure 2 shows the elution profile (UV absorbance, fluorescence and radioactivity) of a 24h urine (incubated with β-glucuronidase and spiked with synthetic reference compounds) from a rat dosed with ^{14}C-NP-coated diesel particles.

DNA and protein were isolated from homogenized lung tissue by $CHCl_3$:iso-amyl alcohol:phenol (24/1/25 by vol.) extraction and were analysed for covalently-bound ^{14}C as described (13).

RESULTS

The rats tolerated the instillation procedures, and the dose of 5 mg of diesel particles, without overt ill-effects. On sacrifice, the lungs of the animals that had received particles were heavier than those of the animals dosed with ^{14}C-NP alone, and appeared slightly oedematous, but no gross lesions were observed. Table 1 shows the overall recovery of ^{14}C 24h after dosing. The pattern of excretion was very similar after all 3 regimens: the majority (65-70%) of the dose was found in the gastro-intestinal tract. The most notable differences observed were the more extensive clearance of ^{14}C from the lung and faster movement of ^{14}C along the gut when ^{14}C-NP was given alone as the pure compound.

Where enough ^{14}C was present to permit extraction and HPLC analysis, most of the material in the lung (75-90%) and stomach contents (86%) was unchanged NP (results not shown). After dosing with the diesel particles, dark particulate matter was observed in the lung, trachea, oesophagus, and stomach contents.

The appearance of ^{14}C along the trachea, oesophagus and in the stomach contents suggests clearance by the mucociliary escalator, of either material still bound to particles (engulphed or not) or material free of the particles but engulphed by macrophages. Studies at shorter time points will be needed to determine how much of the dose is removed from the lung by the mucociliary escalator, compared to absorbtion into the blood.

The urines analysed by HPLC (shown in Figure 2 and Table 2) were similar to each other [and to urines from rats injected intra-peritoneally (12)] in distribution of metabolites, except that rats which had received diesel particles in any form excreted more unconjugated metabolites, and also relatively more 8-OH-NAAP than 6-OH-NAAP, than did rats receiving NP alone.

The lung was the only tissue examined for macromolecular

TABLE 1

DISTRIBUTION OF RADIOACTIVITY IN RATS 24 HOURS AFTER INTRATRACHEAL INSTILLATION OF 1-NITRO[^{14}C] PYRENE (1 - 1.3 x 10^6 DPM) ALONE, CO-ADMINISTERED WITH DIESEL PARTICLES (5 MG) AND VAPOR-COATED ONTO DIESEL PARTICLES (5 MG).

1-Nitro[^{14}C]pyrene instilled:	Alone		Co-Administered With Diesel Particles		Vapor-Coated Onto Diesel Particles	
Tissue[a]						
Lung	< 3.5	(<0.3)	273 ± 68[b,c]	(21.1)	89 ± 8[d]	(8.9)
Stomach contents	24 ± 1[c]	(1.9)	79 ± 73	(6.1)	6 ± 9	(0.6)
Small intestine contents	37 ± 10	(2.9)	58 ± 18	(4.5)	40 ± 13	(4.0)
Large intestine contents	79 ± 32	(6.1)	324 ± 145	(25.1)	201 ± 115	(19.9)
Faeces	772 ± 131	(59.8)	387 ± 70	(30.0)	436 ± 157	(43.2)
Total G.I. tract	912 ± 173	(70.7)	848 ± 15	(65.7)	684 ± 43	(67.7)
Urine	354 ± 19	(27.4)	210 ± 24	(16.2)	271 ± 46	(26.8)
Total recovery	1266 ± 178	(98.1)	1331 ± 76	(103.0)	1043 ± 13	(103.4)

a Other tissues examined, including blood, pancreas, spleen, liver, fat and lymph nodes, did not contain significant ^{14}C counts above background.
b The values given represent total ^{14}C content (x 10^3 dpm) detected in the organ or tissue specified, with the percentage of the dose in parentheses.
c Mean ± S.E.M., n = 2 animals
d Mean ± S.D., n = 3 animals

TABLE 2. DISTRIBUTION OF METABOLITES IDENTIFIED IN 24 HOUR URINE FROM RATS INSTILLED INTRATRACHEALLY WITH 1-NITRO[^{14}C] PYRENE OR ^{14}C-1-NITROPYRENE AND NON-LABELLED DIESEL PARTICLES

Form of Dosage	Metabolite Fraction[a]	Untreated Urine	After incubation with Sulphatase	After incubation with β-glucuronidase
^{14}C-NP alone[b]	SOLV. FRONT	91.4 ± 1.5[b]	83.3 ± 1.5	61.7 ± 1.8
	6-OH-NAAP	1.9 ± 0.2	3.2 ± 0.4	14.5 ± 0.6
	8-OH-NAAP	1.3 ± 0.6	4.0 ± 0.8	6.3 ± 0.1
	DIHYDRODIOL	0.8 ± 0.3	1.9 ± 0.2	6.3 ± 0.8
	3-OH-NAAP	0.4 ± 0.1	0.9 ± 0.1	2.3 ± 0.2
	NAAP	0.7 ± 0.4	3.9 ± 0.1	1.7 ± 0.1
	UNKNOWN I	1.2 ± 0.4	0.9 ± 0.2	2.5 ± 1.5
	1-AP	0.4 ± 0.1	0.5 ± 0.1	2.0 ± 1.4
	6+8-OH-NP	0.3 ± 0.1	0.3 ± 0.1	0.7 ± 0.3
	3-OH-NP	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
	1-NP	0.8 ± 0.3	0.8 ± 0.1	1.1 ± 0.1
	UNKNOWN II	0.5 ± 0.1	0.5 ± 0.1	0.7 ± 0.1
^{14}C-NP Co-Instilled With Unlabelled Diesel Particles[b]	SOLV. FRONT	78.4 ± 5.7	66.8 ± 1.2	50.7 ± 1.3
	6-OH-NAAP	8.0 ± 3.4	7.2 ± 1.2	8.8 ± 0.7
	8-OH-NAAP	3.2 ± 0.6	5.5 ± 1.5	13.1 ± 1.3
	DIHYDRODIOL	2.4 ± 0.5	3.1 ± 1.3	7.9 ± 0.1
	3-OH-NAAP	1.5 ± 0.5	1.7 ± 0.9	2.7 ± 0.8
	NAAP	1.2 ± 0.5	1.6 ± 0.5	2.6 ± 0.9
	UNKNOWN I	0.4 ± 0.1	0.7 ± 0.2	0.8 ± 0.4
	1-AP	0.5 ± 0.1	0.7 ± 0.1	0.8 ± 0.1
	6+8-OH-NP	1.8 ± 0.3	8.4 ± 2.4	8.4 ± 1.8
	3-OH-NP	1.3 ± 0.4	2.7 ± 0.4	2.2 ± 0.8
	1-NP	0.7 ± 0.1	1.1 ± 0.1	1.4 ± 0.6
	UNKNOWN II	0.6 ± 0.2	0.6 ± 0.1	0.7 ± 0.1
^{14}C-NP Vapor-Coated Onto Diesel Particles[c]	SOLV. FRONT	80.5 ± 0.3	69.1 ± 2.5	52.2 ± 5.1
	6-OH-NAAP	6.8 ± 3.1	4.5 ± 0.4	11.1 ± 4.9
	8-OH-NAAP	3.8 ± 0.8	10.1 ± 0.7	14.3 ± 3.8
	DIHYDRODIOL	2.6 ± 0.6	5.3 ± 0.3	8.0 ± 1.9
	3-OH-NAAP	1.0 ± 0.2	1.5 ± 0.2	1.8 ± 0.4
	NAAP	0.8 ± 0.1	1.3 ± 0.2	3.2 ± 0.3
	UNKNOWN I	0.3 ± 0.1	0.6 ± 0.2	0.8 ± 0.1
	1-AP	0.3 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
	6+8-OH-NP	2.1 ± 1.3	5.2 ± 2.3	5.6 ± 1.3
	3-OH-NP	1.1 ± 0.4	1.0 ± 0.4	1.2 ± 0.9
	1-NP	0.4 ± 0.1	0.6 ± 0.1	0.7 ± 0.1
	UNKNOWN II	0.3 ± 0.1	0.5 ± 0.1	0.4 ± 0.1

a Metabolite fractions correspond to those in Figures 1 and 2.
b The values given are means ± S.E.M. from 2 rats per group.
c The values given are means ± S.D. from 3 rats per group.

TABLE 3

INTRA-PULMONARY DISTRIBUTION OF ^{14}C 24 H AFTER DOSING

Tissue	Form of dosage:	NP alone	NP + Diesel Particles	NP Vapor-Coated Onto Diesel Particles
Trachea:	Total ^{14}C[a]	417 ± 14	616 ± 100	767 ± 489
	% of dose	0.03	0.05	0.08
Lung Lavage:	Supernatant[a]	<2 000	<4 000	< 1 750
	% of dose	< 0.15	< 0.3	< 0.2
	Macrophages[a]	< 250	21 992 ± 9 398	10 787 ± 8 823
	% of dose	< 0.02	1.70	1.07
Lung homogenate:	Total ^{14}C[a]	<3500	250 468 ± 77 054	78 313 ± 12 754
	% of dose	< 0.3	19.38	7.76
Lung Protein:	Total ^{14}C[a]	< 300	15 283 ± 7 212	5 817 ± 1 837
	% of dose	< 0.03	1.18	0.58
	Binding level[b]	< 0.9	33.4 ± 13.6	12.5 ± 3.1
Lung DNA:	Total ^{14}C[a]	< 450	< 450	<120
	Binding level[c]	< 0.5	< 0.5	< 0.4

a Total ^{14}C content (in d.p.m.) was determined by liquid scintillation counting.
b Binding is expressed as pmol/g wet weight tissue.
c The lowest detectable levels of binding are expressed as pmol/mg DNA.

binding as it was the only organ containing significant amounts of ^{14}C (Table 3). No ^{14}C was bound to DNA, within our limits of detectability of 0.5 pmol per mg DNA. After exhaustive extraction and re-precipitation, 5 800 d.p.m., representing 0.6 % of the dose, remained associated with the protein fraction from the lungs of rats dosed with ^{14}C-NP vapor-coated onto diesel particles. After co-instillation of ^{14}C-NP with unlabelled diesel particles, 15 000 d.p.m. (1.2 % of the dose) was recovered in the protein fraction. Virtually no residual protein-bound ^{14}C was found in the lungs from rats gived ^{14}C-NP alone.

DISCUSSION

The overall excretion pattern of ^{14}C seemed similar after intratracheal instillation of ^{14}C-NP either coated onto diesel particles, co-instilled along with unlabelled diesel particles, or administered alone, and was also similar to that seen after intraperitoneal injection of pure ^{14}C-NP (10). The profile of urinary metabolites seen 24 hours after dosing with the diesel particles differed from that obtained from the pure compound alone mainly in that a smaller proportion of metabolites were excreted unconjugated. The radioactivity instilled into the trachea was rapidly cleared, from the lung either by absorption into the blood or via the mucociliary escalator into the gastro-intestinal tract, except for that portion tightly associated with the lung protein. The lung and the gastro-intestinal tract would thus appear to be the organs most exposed to NP deposited on inhalable particles, although this does not mean that they would necessarily be the sole target organs for toxic or genotoxic damage arising from such exposures. Conflicting evidence from the in vivo studies reported to date (5,6,17) suggests that the occurrence and site of carcinogenicity of NP may depend on the species and route of administration. Thus tumorigenicity of extracts of diesel emissions in Sencar mice (18) cannot be attributed to NP, since this compound was inactive in similar mouse skin-painting studies (17). The occurrence of mammary gland tumours (6) indicates that NP may require specific routes of metabolism to undergo activation.

The similarity in percentage of dose excreted in urine and in the resultant urinary metabolite profile following different dosages of NP on, off, or associated with, diesel particles confirms previous indications (15) that this compound is readily bioavailable from diesel particles. The further similarities with the fate of a one-thousand-fold larger dose of ^{14}C-NP (8 µmoles) injected intraperitoneally (10) indicate that its

metabolic routes (including enterohepatic recirculation) are likely to be similar over a wide range of dose levels. The doses of diesel particles used in this study (5 mg, 20 mg/kg) correspond to the total amount inhaled (although not necessarily deposited) by a rat breathing air contaminated with 600μg/m^3 particulate for 661 hours (19). A human breathing the same air would, by virtue of a greater lung capacity (20), inhale this dose in 20 hours. Since passenger diesel automobiles emit from 200 to 500 mg particulate/mile driven/car (21), the dose used in this study could be expected to fall within the range of local concentrations found on the highway. The NP level on the particles also lies within the reported values for this compound on various diesel emission samples (3,16,21).

Protein-bound ^{14}C was detected in particle-treated lungs, though the divergence in species and treatment precludes direct comparison with the levels previously formed by NP in vitro(13). No such DNA adducts were found, although the specific activity of the ^{14}C-NP used would have allowed detection of 0.5 pmol bound/mg DNA, compared to 35 pmol/mg DNA noted in the previous study (13). Adducts may nevertheless be formed in vivo, but at levels much lower than in vitro, which would require NP of higher specific activity for detection and quantitation.

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REFERENCES

1. Löfroth, G., Hefner, E., Alfheim, I., and Møller, M. (1980): Mutagenic activity of photocopies, Science, 209: 1037-1039.

2. Rosenkranz, H.S., McCoy, E.C., Sanders, D.R., Butler, M., Kiriazides, D.K., and Mermelstein, R. (1980): Nitropyrenes: Isolation, identification and reduction of mutagenic impurities in carbon black and toners, Science, 209:1039-1043.

3. Schuetzle, D., Riley, T.L., Prater, T.J., Harvey, T.M. and Hunt, D.F. (1982): Analysis of nitrated polycyclic hydrocarbons in diesel particulates, Analyt. Chem., 54:265-271.

4. Mermelstein, R., Kiriazides, D.K., Butler, M., McCoy, E. and Rosenkranz, H.S. (1981): The extraordinary mutagenicity of nitropyrenes in bacteria, Mutat. Res., 89: 187-196.

5. Ohgaki, H., Matsukara, N., Morino, K., Kawachi, T., Sugimura, T., Morita, K., Tokiwa, H., and Hirota, T. (1982): Carcinogenicity in rats of the mutagenic compounds 1-nitropyrene and 3-nitrofluoranthene, Cancer Lett., 15: 1-7.

6. Hirose, M., Lee, M.S., Wang, C.Y., and King, C.M. (1984): Induction of rat mammary gland tumors by 1-nitropyrene, a recently-recognized environmental mutagen, Cancer Res., 44:1158-1162.

7. Messier, F., Lu, C., Andrews, P., McCarry, B.E., Quilliam M.A., and McCalla, D.R. (1981): Metabolism of 1-nitropyrene and formation of DNA adducts in Salmonella typhimurium, Carcinogenesis, 2:1007-1011.

8. Howard, P.C., Heflich, R.H., Evans, F.E., and Beland, F.A. (1983): Formation of DNA adducts in vitro and in Salmonella typhimurium upon metabolic reduction of the environmental mutagen 1-nitropyrene, Cancer Res., 43:2052-2058.

9. El-Bayoumy, K., and Hecht, S.S. (1983): Identification and mutagenicity of metabolites of 1-nitropyrene formed by rat liver, Cancer Res., 43:3132-3137.

10. Ball, L.M., Kohan, M.J., Claxton, L.D., and Lewtas, J. (1984): Mutagenicity of derivatives and metabolites of 1-nitropyrene: Activation by rat liver S9 and bacterial enzymes, Mutat. Res., (in press).

11. Ball, L.M., Rafter, J.J., Gustafsson, J.A., Gustafsson, B., and Lewtas, J. (1983): Metabolism of 1-nitropyrene in germ-free and conventional rats: role of the gut flora in generation of mutagenic metabolites. In: Extrahepatic

Drug Metabolism and Chemical Carcinogenesis, edited by Rydström, J. et al, pp 295-296. Elsevier Press, Amsterdam.

12. Ball, L.M., Kohan, M.J., Inmon, J., Claxton, L.D., and Lewtas, J. (1984): Metabolism of 1-nitro[^{14}C]pyrene in vivo in the rat and mutagenicity of urinary metabolites. Carcinogenesis, 5:(in press).

13. King, L.C., Jackson, M., Ball, L.M. and Lewtas, J. (1983): Binding of 1-nitro[^{14}C]pyrene to DNA and protein in cultured lung macrophages and respiratory tissues, Cancer Lett, 19:241-246.

14. Jackson, M.A., King, L.C., Ball, L.M., Ghayourmanesh, S., Jeffrey, A.M., and Lewtas, J. (1984): Nitropyrene: DNA binding and adduct formation in respiratory tissues, Environ. Hlth. Perspect., (in press).

15. King, L.C., Kohan, M.J., Austin, A.C., Claxton, L.D., and Lewtas, J. Huisingh (1981): Evaluation of the release of mutagens from diesel particles in the presence of physiological fluids, Environ. Mutag., 3:109-121.

16. Tejada, S.B., Zweidinger, R.B., and Sigsby, J.E. (1984): Aanlysis of nitroaromatics in diesel and gasoline car emissions, SAE Technical Paper Series, #820775, Troy, MI, June 7-9, 1982.

17. Nesnow, S., Slaga, T., and Triplett, L. (1984): Tumor-initiating activity of 1-nitropyrene and its nitrated products in Sencar mice, Cancer Lett., 23:1-8.

18. Nesnow, S., and Lewtas, J. (1981): Mutagenic and carcinogenic potency of extracts of diesel and related environmental emissions: Summary and discussion of the results, Environ. Internat., 5:425-429.

19. Inglis, J.K. (1980): Introduction to Laboratory Animal Science and Technology. Pergamon Press, Oxford. (Cited in Harvard Bioscience Whole Rat Catalog, 1983, pp vi-vii.)

20. Ganong, W.F. (1973): Review of medical physiology (Sixth Edition). Lange Medical Publications, Los Altos, CA, 578 pp.

21. Lewtas, J. (1983): Evaluation of the mutagenicity and carcinogenicity of motor vehicle emissions in short-term bioassays, Environ. Hlth. Perspec., 47:141-152.

IN VIVO METABOLISM OF PARTICLE-ADSORBED 1-NITRO[^{14}C]PYRENE

KEY WORDS:

Instrumentation: HPLC
Major Chemical Species Studied: 1-Nitropyrene, Diesel Particles
Animal Species: Rat
Unique Radiolabelled Materials: 1-Nitro[^{14}C]pyrene, 1-nitro-[^{14}C]pyrene vapor-coated onto diesel particles.
Macromolecules: Protein, DNA
Nucleic Acids: DNA
Measurement Endpoints: ^{14}C content, metabolite formation, protein binding, DNA binding
Other: Metabolism, distribution